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Mechanical Signalling in Osteoarticular Tissues

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Within a physiological range exposure to mechanical loads is required to maintain bone, cartilage and associated connective tissues in a healthy state. Conversely withdrawal of these mechanical stimuli or overloading can lead to connective tissue breakdown and degenerative diseases. Over the last few decades there has been a large increase in our knowledge of how mechanical forces are recognised by connective tissue cells and how these forces regulate cell function and tissue homeostasis. Mechanical forces applied to connective tissues are recognised by cell mechanoreceptors which then activate signal cascades resulting in altered gene expression, protein production and tissue remodelling. The nature of these mechanoreceptors, the signalling pathways and secreted paracrine/autocrine molecules that further regulate the process are discussed.

Key words: cartilage, mechanoreceptors, osteoarthritis

INTRODUCTION

Connective tissues including bone^{1,2}, cartilage³, tendon⁴ and skeletal muscle require exposure to mechanical forces to remain in a healthy state. The impact of mechanical forces on bone and cartilage is seen early in life where it is essential for normal embryonic development and morphogenesis of the skeleton and joints. The response of adult tissues was epitomised by Wolff's observations on bone. He observed that in a healthy person or animal bone will adapt to the loads it is placed under. An increase in load leads to bone remodeling with the bone becoming stronger to resist that sort of loading. When loading decreases, the bone will atrophy as there is no stimulus for continued remodeling that is required to maintain bone mass. The response to mechanical stresses in most connective tissues can be considered as having a bell shaped distribution with too little loading resulting in atrophy and excess mechanical stimulation leading to tissue damage. The ability to alter structure in response to mechanical loading is termed tissue mechanical adaptation.

Within a physiological range, mechanical loading of connective tissues that occurs as a result of normal, everyday activity, is sufficient to keep connective tissues healthy. Physical exercise where increased loads are applied increases connective tissue performance. Such changes are clearly seen in athletes where skeletal muscle bulk and strength increases but other connective tissues are similarly affected. Tennis players who start training before puberty have increased bone mass in dominant arms⁵. Exercise increases proteoglycan synthesis and articular cartilage thickness⁶. With exercise the mechanical strength and collagen content of tendons rises⁷. The requirement for mechanical loading to maintain connective tissue is evident in paraplegics, patients on prolonged bed rest and in astronauts. The bones in paralysed and under-used limbs are architecturally and mechanically inferior, immobilized tendons lose mechanical strength⁸. Overloading and under loading of articular cartilage result in loss of cartilage matrix and development of osteoarthritis⁹.

MOST CONNECTIVE TISSUE CELLS ARE MECHANOSENSITIVE

Adaptation in response to mechanical loading requires that cells within connective tissue receive information from the environment. Cells exposed to mechanical loads modify the environment, essentially the connective tissue composition, so that it is appropriate for the new range of mechanical stresses to which it is now exposed. This pro-

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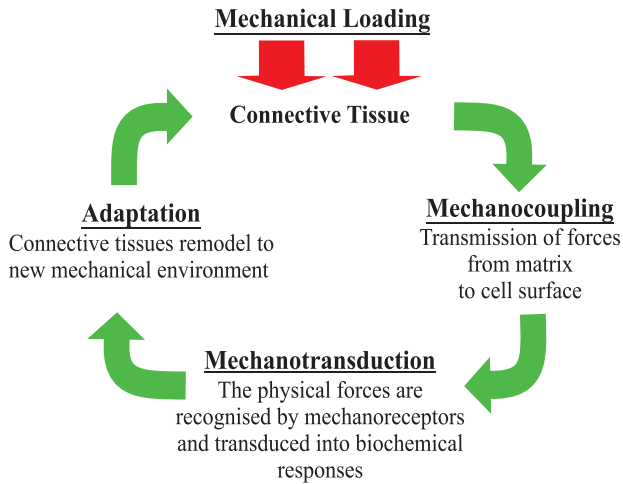


Fig. 1 Response of connective tissues to mechanical loading.

cess has a number of phases¹⁰. Forces at the macroscopic level are translated through the extracellular matrix (Fig. 1) to the mechanosensitive cell - mechanocoupling. The mechanical forces at the cell membrane of the mechanosensitive cell are then transduced into biochemical signals within the cell - mechanotransduction. Effector cells, most commonly but not exclusively the mechanosensitive cells, initiate tissue modelling/remodelling. Most connective tissue cells appear to be mechanosensitive. In cartilage chondrocytes sense mechanical stimuli and as effector cells produce both matrix macromolecules and proteases required for tissue turnover and homeostasis. Similarly the tenocyte appears to have a major role in mechanical tissue adaptation in tendons¹¹. In bone tissue remodelling involves complicated interactions between progenitor cells, endothelial cells, osteoclasts and bone forming cells. Osteocytes appear to be the most important mechanosensitive cell in bone stimulating bone remodelling through paracrine effects on osteoprogenitor cells and osteoclasts^{1,2}.

MECHANOCOUPLING

Bone, cartilage, tendon and ligaments are exposed to a range of mechanical forces *in vivo*. The loads encountered by cartilage and tendon are exposed much higher than that of bone¹². Forces of three to seven times body weight are generated in the hip and knee on with normal to rapid walking. Forces are applied rapidly with up to 20 MPa being produced in the hip within milliseconds on standing or climbing stairs. The achilles tendon may

encounter forces of four to twelve times body weight during walking and running¹³. Normal activity appears to result in strains of only 10 microstrain at the surface of bone although this can rise 300 folds during vigorous exercise.

Of course connective tissue cells are not directly exposed to these mechanical forces. The surrounding extracellular matrix is protective and acts to absorb, transmit and dissipate these forces and the forces perceived by the cells are expected to be much smaller. Indeed the nature of the mechanical signal that is finally sensed by the cell is not entirely clear and may be the summation of a number of different changes in the physicochemical environment. In cartilage dynamic compression induces hydrostatic pressure gradients, fluid flow, streaming potentials, alterations in matrix water content, fixed charge density, mobile ion concentrations, and changes in pH and osmotic pressure¹⁴ as well as chondrocyte deformation. Loading of bone generates up to 5000 microstrain at the osteocyte cell surface and pressure gradients that stimulate interstitial fluid flow through the osteocytic lacunar network^{15,16}. Tensile loads at the tissue level are converted into tensile, shear and compressive strains perceived by tendon cells¹⁷.

MECHANOTRANSDUCTION

The physiochemical changes, including changes in cell shape, cell membrane deformation and fluid flow that occur within connective tissue during mechanical loading are recognised by cell mechanoreceptors. Mechanoreceptor activation stimulates a variety of intracellular cell signalling cascades that regulate gene expression, changes in protein expression and tissue remodelling. Integrins, mechanosensitive ion channels, and connexins have each been identified as candidate mechanoreceptors¹⁸⁻²¹.

Integrins

Integrins are heterodimeric transmembrane glycoproteins comprising α and β subunits. Each α and β subunit has an extracellular domain, a single transmembrane region, and a cytoplasmic tail. The extracellular domain provides the ligand-binding site for matrix molecules. The cytoplasmic tail is coupled to both the cytoskeletal network and signalling molecules such as focal adhesion kinase (FAK) allowing transduction of mechanical signals transmitted through the matrix into biochemical responses within the cell²². Integrins mediate cellular responses to stretch, elevated hydrostatic pressure, fluid shear stress, and osmotic forces²³. The mechanisms of

integrin dependent mechanotransduction are being clarified. Stretch may increase tension and activate integrins that are bound to the extracellular matrix. Subsequent activation of a subset of unoccupied integrins inducing their binding to matrix ligands proteins and stimulation of intracellular signalling can amplify the signal. Other mechanical forces probably act similarly, increasing activation of integrins to stimulate cell adhesiveness and signalling. The cytoskeleton also responds mechanically to forces channelled through integrins by rearranging its interlinked actin microfilaments, microtubules and intermediate filaments²⁴.

A variety of integrins, many of which may function as mechanoreceptors, are expressed by chondrocytes and bone cells. Roles for $\alpha_5\beta_1$, $\alpha_V\beta_5$ and $\alpha_V\beta_3$ have been demonstrated *in vitro* but $\alpha_5\beta_1$ integrin, the classical fibronectin receptor, appears to be the major integrin mechanoreceptor in connective tissues. The anabolic response of human articular chondrocytes to mechanical stimulation is $\alpha_5\beta_1$ dependent and involves phosphorylation of focal adhesion proteins including FAK and paxillin²⁵. Integrins may be part of larger mechanoreceptor complexes including accessory molecules such as CD47 that may control integrin activation²⁶. Roles for α_1 integrins as osteocyte mechanoreceptors have been demonstrated in a conditional knock out model where absence of the α_1 integrin limits the response of bone to disuse²⁷.

Stretch-activated Ion channels

Stretch-activated or stretch-sensitive ion channels (SACs) open as a consequence of mechanical deformation of the cell membrane²⁰. SACs are directly activated by mechanical forces applied along the plane of the cell membrane that induce membrane tension and distortion of the lipid bilayer. These result in conformational changes which alter opening or closing rates of the channels permitting ion flux²⁴. Application of mechanical forces perpendicular to the cell membrane, as seen with hydrostatic pressure, appears to be less effective in activating SACs²⁰. Activation of calcium permeable SACs leads to local increase in intracellular calcium levels and stimulation of downstream calcium-dependent intracellular signal cascades. SACs sensitive to gadolinium are necessary for load and fluid flow related cellular responses in both chondrocytes and bone cells.

Connexins

Connexins are widely expressed in connective tissue where networks of cells are seen such as in bone, tendon and meniscus. They probably act to allow propagation

of a mechanical stimulus through a tissue. They are a superfamily of twenty-one transmembrane proteins that form gap junctions and hemichannels²¹. Gap junctions allow continuity between cells permitting diffusion of ions, metabolites and small signaling molecules such as cyclic nucleotides and inositol derivatives. Cx43 is the most abundant connexin present in skeletal tissue. Conditional deletion of reduces mineral apposition rate to mechanical loading and Cx43 hemichannels are important for fluid shear induced PGE₂ and ATP release in osteocytic cells²⁸. Connexins and gap junctions are present at the tip of osteocyte dendritic processes and between these processes and osteoblasts indicating their potential importance in permitting cell-cell communication among the osteocytic network although propagation may be only directed from osteocytes to osteoblasts²⁹. Cx32 and Cx43 are important in tenocyte mechanotransduction³⁰. Cx32 junctions form a communication network arranged along the line of principal loading and stimulate collagen production in response to strain. In contrast the Cx43 network links tenocytes in all directions and is inhibitory for collagen production. In cartilage the importance of connexins for responses to mechanical loads is less clear but may have roles as part of the primary cilia.

Primary Cilia

Primary cilia are solitary, immotile cilium present in most cells including chondrocytes and bone cells. They are microtubule-based organelles, growing from the centrosome to extend from the cell surface and contain large concentrations of cell membrane receptors, including integrins. They function both as chemosensors and mechanosensors^{31,32}. Bending of the cilium upon matrix deformation or with fluid flow is thought to cause cilium bending, pulling on associated matrix receptors and activation of the mechanoreceptors³³. In addition to integrins, Cx43 hemichannels are also present on primary cilia and by regulating ATP release cilia and activation of purine receptors cilia-associated connexins may also be involved in mechanotransduction.

INTRACELLULAR SIGNAL CASCADES ACTIVATED BY MECHANICAL SIGNALS

Stimulation of connective tissue cell mechanoreceptors is followed by generation of the secondary messenger molecules and activation of a cascade of downstream signalling events that regulate gene expression and cell function. Many intracellular signalling pathways are known to be activated by mechanical forces applied to

tissues and cells including heterotrimeric guanine nucleotide binding proteins (G-proteins), protein kinases and transcription factors. These pathways that regulate tissue modelling/remodelling may be activated directly as a consequence of mechanoreceptor signalling or indirectly following production of autocrine/paracrine acting molecules.

The phosphoinositide3 kinase –Akt/Protein kinase B (PKB) pathway

PKB/Akt is a protein family of serine/threonine kinases that have multiple roles including inhibition of apoptosis by phosphorylation and inactivation of pro-apoptotic factors. Integrin-dependent activation of phosphoinositide3 kinase (PI3 kinase) by mechanical forces regulates PKB activity and can inhibit cell death. Inactivation of the PI3-K/PKB pathway may be important in deleterious effects of mechanical overloading of cartilage and bone loss in response to withdrawal of loading³⁴.

Mitogen-activated protein kinases

Mitogen-activated protein (MAP) kinases regulate multiple cellular activities, such as gene expression, mitosis, differentiation, and cell survival/apoptosis. ERK1/2, JNK and p38, of critical importance in regulation of matrix protein and protease gene expression have each been shown to be activated in chondrocytes, bone cells and fibroblasts^{2,35,36} following mechanical stimulation. Different mechanical stimuli may activate different MAPKs and through this mechanism differential cellular responses may occur. MAPK responses may also be cell type dependent. Mechanical stimulation induced ERK1/2 activation in bone cells requires calcium-dependent ATP release whilst in cartilage activation, under certain circumstances, is dependent on FGF-2 rather than through integrin mechanoreceptors³⁷.

The NF-kappa B pathway

In bone cells NF- κ B, a protein complex that acts as a transcription factor, is directly stimulated by mechanical stimulation is dependent on intracellular calcium release³⁸. In chondrocytes biomechanical signals within the physiological range block NF- κ B activity and proinflammatory chondrocyte responses³⁹. Mechanical stimuli that induce catabolic rather than anabolic responses in chondrocytes induces rapid nuclear translocation of NF- κ B subunits p65 and p50 in a similar manner to IL1 β .⁴⁰

GROWTH FACTORS AND AUTOCRINE / PARACRINE SIGNALING EVENTS IN MECH-

ANOTRANSDUCTION

As part of the cellular response to mechanical stimulation mechanosensitive connective tissue cells release a range of soluble mediators. These may be present in the cell and available for immediate release, or secretion may depend de novo synthesis by enzymatic activity or transcriptional activation and protein production. These mediators, including prostaglandins, nitric oxide, cytokines, growth factors, and neuropeptides are involved in downstream tissue modelling and remodelling responses initiated by the mechanosensitive cell or other effector cells. Production of soluble mediators by connective tissue cells in response to mechanical stimulation however may also be intrinsic to mechanotransduction pathways. Autocrine and paracrine activity allows increased regulation of the cellular response to mechanical stimuli by permitting cross talk between different components of a mechanotransduction cascade. As the cellular responses to mechanical stimuli and soluble mediators activate similar signal cascades inducing either anabolic or catabolic responses, it would be expected that they may be antagonistic, additive or synergistic. Anabolic cytokines and growth factors enhance production of matrix under mechanical loading conditions whilst anabolic mechanical stimuli antagonise the effects of catabolic cytokines such as IL1 β .⁴¹

Prostaglandins, Nitric Oxide and Adenosine triphosphate (ATP)

Prostaglandins, predominantly PGE₂, NO and ATP are synthesised and released when bone cells and chondrocytes are mechanically stimulated. Both PGE₂ and NO are required for the anabolic response of bone to mechanical loading *in vivo*. Prostaglandin production is integrin dependent requiring an intact cytoskeleton and activation of SACs, PKC, and PLA₂. PGE₂ may influence responses to mechanical loading by regulating osteocyte gap junction expression⁴², RANKL expression and osteoclastogenesis. In cartilage PGE₂ induced by mechanical loads is catabolic. Mechanical loading of chondrocytes by physiological stimuli inhibits production of PGE₂ and NO whereas damaging high impact loading induces PGE₂ release⁴³. Following mechanical stimulation bone cells and chondrocytes release ATP which can bind and activate purinergic receptors on these and adjacent cells. Both metabotropic P2Y receptors and ionotropic P2X receptors, have been shown to be involved in mechanical load activated signal cascades in chondrocytes and bone cells and may have physiological roles. Osteopaenia, as a

result of P2X₇ deficiency is potentially a result of reduced responsiveness to mechanical loading of the skeleton⁴⁴.

Cytokines and Growth Factors

Interleukin 4 (IL4) and interleukin 1 (IL1) auto-crine/paracrine activity is seen in the integrin-dependent mechanotransduction cascade of chondrocytes (IL4 and IL1) and bone cells (IL1) to mechanical stimulation⁴⁵. These molecules are secreted within 20 minutes of the onset of mechanical stimulation, suggesting release from preformed stores. IL4 release relies on secretion of the neuropeptide substance P which binds to its NK1 receptor. Both IL4 and substance P are necessary but not sufficient for the increased expression of aggrecan mRNA and decrease in MMP3 mRNA induced by the mechanical stimulus suggesting cross talk with other mechanosensitive signaling pathways. IL1 is involved in the early mechanotransduction pathway of both osteoarthritic chondrocytes and human trabecular bone derived cells^{46,47}. Mechanical loading may also induce release or activation of sequestered growth factors in extracellular matrix which will then act on near-by resident connective tissue cells. Basic fibroblast growth factor (FGF2) is a possible mediator of mechanical signalling in cartilage through such a mechanism³⁷. Dynamic compression of porcine cartilage induces release of FGF2 with activation of ERK MAP kinase, synthesis and secretion of tissue inhibitor of metalloproteinases 1 (TIMP-1). In contrast FGF2 production by bovine cartilage is inhibited by 1 hour of compressive stress of 20 MPa⁴⁸. This mechanical induced suppression of FGF2 is blocked IL4 indicating further roles for this pleiotropic cytokine in the regulation of chondrocyte responses to mechanical stimulation.

SUMMARY

Connective tissues face a wide range of mechanical stresses during daily activity. Many of these will act to maintain connective tissues, including bone cartilage and tendons in a healthy state. Moderate exercise can improve the strength and function of connective tissues but withdrawal of mechanical stimuli, acute mechanical injury and chronic overloading leads to tissue damage and diseases such as osteoarthritis and osteoporosis. Acute mechanical trauma will directly disrupt the collagenous network of connective tissues by physical means but will also stimulate a variety of cellular changes including activation of catabolic and pro-apoptotic pathways.

Increased knowledge of mechanoreceptors and mecha-

notransduction pathways are informing on how mechanical stresses regulate connective tissue homeostasis. New insights as to how adverse mechanical environments have detrimental effects on connective tissue should allow development of novel therapeutic interventions such as mechanomimetics to preserve or reconstitute connective tissue in ageing and disease.

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